bio-protocol Preprint

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Animal husbandry and handling

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An abbreviated version of this protocol was published in eLIFE in Mar 2020

Losing Dnmt3a dependent methylation in inhibitory neurons impairs neural function by a mechanism impacting Rett syndrome

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Detailed protocol

For genotyping R26-CAG-LSL-Sun1-sfGFP-Myc+/+ we used a modified version of the JAX protocol. However, this genotyping only tells you that the transgene is there, and if it is homozygous or heterozygous. I have not seen any protocol for genotyping to detect if the LSL is recombined, though it should be possible to design if one had the transgene sequence. This may be available through Jeremy Nathans' group at Johns Hopkins, who developed the mice.
For our genotyping, we monitored recombination using the other allele (Dnmt3a flox/flox). In this genotyping scheme we can detect the recombined allele.
Here is the genotyping scheme used for the R26-CAG-LSL-Sun1-sfGFP-Myc+/+ allele (again without the ability to see LSL status). Still this may be useful as it is a single reaction as opposed to 2 separate proposed by JAX.
Primers:

JAX_16014_Common	GCACTTGCTCTCCCAAAGTC
JAX_16015_WT_R	CATAGTCTAACTCGCGACACTG
JAX_8820_Mut_R	GTTATGTAACGCGGAACTCC

Mix primers with 20uM 16014 + 10uM each 16015 and 8820

For a 1X reaction:
6uL EcoTaq (or equivalent)
5uL H2O
1uM primer mix
1.5uL Tail lysis/DNA

Cycling
95C 5min
94C 30sec
56C 40sec
72C 1min
go to step 2 34 times
72C 5min
4C forevver

Band size:
WT = 500bp
Transgene = 300bp

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Lavery, L. and Zoghbi, H. Y.(2021). Animal husbandry and handling. Bio-protocol Preprint. bio-protocol.org/prep1054.
2. Lavery, L. A., Ure, K., Wan, Y., Luo, C., Trostle, A. J., Wang, W., Jin, H., Lopez, J., Lucero, J., Durham, M. A., Castanon, R., Nery, J. R., Liu, Z., Goodell, M., Ecker, J. R., Behrens, M. M. and Zoghbi, H. Y.(2020). Losing Dnmt3a dependent methylation in inhibitory neurons impairs neural function by a mechanism impacting Rett syndrome. eLIFE. DOI: 10.7554/eLife.52981

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